Journal of Animal Research: v.7 n.4, p. 785-788. August 2017

DOI: 10.5958/2277-940X.2017.00120.6

SHORT COMMUNICATION

Genotypic Study of Verocytotoxic *E. coli* in Cattle by Multiplex Polymerase Chain Reaction

Raghavendra Prasad Mishra[1], Udit Jain[1], Barkha Sharma[2], Saraswati Ojha[3], Savyasanchi Tripathi[4] and Anita Maroti Chappalwar[3]

[1]Department of Veterinary Public health, DUVASU, Mathura, Uttar Pradesh, INDIA
[2]Department of Veterinary Epidemiology and Preventive Medicine, DUVASU, Mathura, Uttar Pradesh, INDIA
[3]Department of Livestock Products Technology; (DUVASU), Mathura, Uttar Pradesh, INDIA
[4]Department of Veterinary Public health, LUVAS, Hisar, INDIA

Corresponding author: RP Mishra: Email: rmishra523@rediffmail.com

Received: 30 March, 2017 **Revised:** 19 June, 2017 **Accepted:** 20 June, 2017

ABSTRACT

E. coli is the most commonly observed gastrointestinal flora of animals and environmental contaminant considered as important food borne pathogen causing serious complications in man and animals. The study was undertaken to detection of virulence gene using polymerase chain reaction (PCR) in cattle. In present study, a total of 160 samples were processed for isolation of verocytotoxic *E. coli* (VTEC). All samples were positive for *Escherichia coli*. Out of 160 *Escherichia coli*, 14 samples were found positive for VTEC. Out of 14 VTEC, 6 were found to be positive for stx_1 gene (180 bp), 5 were stx_2 (255 bp) and 3 were for stx_1, stx_2 and *hlyA* gene.

Keywords: Fecal sample, *Vercytotoxic E. coli,* PCR

In human beings diarrhoea is one of the most common and multifactorial disease mainly caused by *E. coli* (Kumar *et al.,* 2013). *E. coli* is the most commonly observed gastrointestinal flora of animals and environmental contaminant considered as important food borne pathogen causing serious complications in man and animals (Mailk *et al*., 2013; Dhama *et al.,* 2013; Anita *et al.,* 2014). VTEC is also termed as shiga-like toxin producing *E. coli* (SLTEC) or shiga toxin producing *E. coli* or STEC. Acronym STEC is derived from the fact that the toxins are shiga like that is similar to those produced by *Shigella dysenteriae* type1 (Brien *et al.,* 1987). The adherence factors (intimin) enables the organism to attach to and colonize intestinal mucosal cells (Hiruta *et al.,* 2001). Among VTEC, serotype O157:H7 has been closely associated with the sporadic and clinical outbreaks of hemorrhagic colitis (HC), haemolytic uremic syndrome (HUS) and thrombotic thrombocytopenic purpura (TTP) in human beings (Croxen and Finlay, 2010; Gyles and Fairbrother, 2010; Sanchez *et al.,* 2013). Healthy domestic ruminants are recognised as the main natural reservoir of STEC and large game animal may be healthy carriers of STEC (Diaz *et al.,* 2011; Sanchez *et al.,* 2010).

MATERIALS AND METHODS

Sampling and isolation of *E. coli*

A total 160 randomly selected fecal samples of cattle was taken for the detection of virulent genes of VTEC. The samples were collected aseptically in UV sterile polythene bags and immediately transported to the laboratory under chilled conditions for microbiological analysis. For primary isolation of *E. coli* (VTEC), 10gm of faecal sample were enriched in 90 ml modified trypticase soya broth (mTSB) containing acriflavine (10 mg/l) to reduce the growth of gram positive organism. The method used for collection of materials, and isolation and identification

techniques were made as per the lines suggested by World organization for Animal Health (OIE, 2014). These samples were incubated at 37°C for 6 h. MacConkey's Agar (MCA) was used as differential media, while eosin methylene blue (EMB) agar (Hi-Media, India) was used as selective media. Suspected *E. coli* strains were subjected to morphological, cultural and biochemical characterization as per the standard method (Ewing, 1986).

Molecular characterization

Multiplex Polymerase chain reaction (PCR) was used for detection of virulent genes (stx_1, stx_2, *eaeA* and *hlyA*) of VTEC. All the *E. coli* isolates were subjected to genomic DNA isolation. The bacterial growth in TSB broth (HiMedia) was centrifuged at 3000 rpm for 15 min to make the pellet of bacterial cells. These cells were washed twice with PBS (pH 7.4) to remove any impurity of broth media. Bacterial DNA was extracted by using DNA extraction kit (Genei, Bangalore) as per the manufacturer's protocol. For the PCR reaction, PCR Master Mix solution (Genei, Bangalore) was used. To amplify DNA targeted to virulent genes (stx_1, stx_2, *eaeA* and *hlyA*) of VTEC by using primers on 3µl of DNA template in 25µl reaction mixture (Paton and Paton, 1998). After an initial denaturation step at 95 ºC for 4 min, 30 amplification cycles were performed, each consisting of 94°C for 2 min.,65 °C for 2 min. and 72 °C for 1.5 min and followed by a final extension step at 72 ºC for 2.5 min. After the amplification, amplicons were separated in 1.5% gel in tris acetate EDTA (TAE) buffer at 60 volt for 80 min, stained with 0.5% ethidium bromide solution and visualized under ultraviolet light.

RESULTS AND DISCUSSION

Out of 160 fecal sample, a total of 160 *E. coli* strains were obtained. All the strains of *E. coli* were screened to detect the presence of VTEC genes using multiplex PCR (Fig. 1). An overall prevalence of VTEC in Cattle fecal sample was found to be 8.75% (14/160). Out of 14 VTEC, 6 was found to be positive for stx_1 gene (180 bp), 5 was stx_2 (255 bp) and 3 was for stx_1, stx_2 and *hlyA* gene (255 bp and 534 bp). In present study prevalence of VTEC was 8.75% which showed similar finding to previous study, the prevalence of VTEC in cattle were reported as 8.9% (Eriksson *et al.,* 2005). However, prevalence of VTEC in higher level was reported by previous workers as prevalence of VTEC was 16.66% (Parul *et al.,* 2014), 18% (Rogerie *et al.,* 2001) and 18.47% (Rabin, 1994). In contrast, investigations have shown a higher detection rate of 46% (Kobayashi *et al.,* 2001) in fecal samples of cattle. Lower isolation rate of VTEC in cattle i.e. 9% (Blanco *et al*., 1993). Prevalence rate as low as 1.0% has also been reported (Khurana and Kumar, 2005). VTEC possessed the stx_2 gene, either alone (22 of 32 isolates, 68.8 percent) or with stx_1 (eight isolates, 25.0%) (Wieczorek *et al*., 2011).

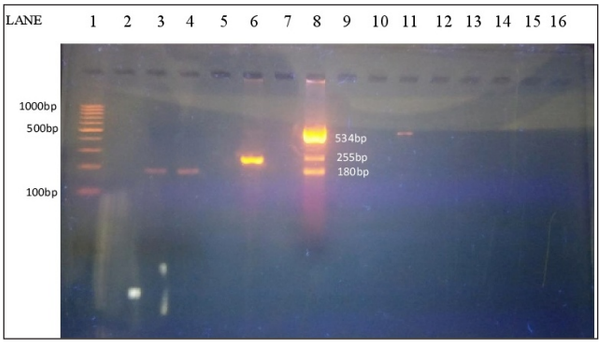

Fig. 1: Detection of the presence of VTEC genes using Multiplex PCR, isolated from fecal sample of Cattle. **Lane 1:**100bp DNA Ladder, **Lane 3,4:** Stx_1 (180bp), **Lane 6:** Stx_2 (255bp) and **Lane 8:** Stx_1, Stx_2 *and hlyA*(534bp)

The close association with ruminants, low infective dose and unusual acid tolerance, have made VTEC a serious global zoonotic problem of great public health significance. VTEC can be present in the intestinal tract of a wide range of domestic and wild ruminants (cattle, buffalo, sheep, goats and deer) (Beutin *et al.,*1995; Johnson *et al.,*1996; Schouten *et al.,* 2005; Oporto *et al.,* 2008).

CONCLUSION

The presence of VTEC genes in feces of a large population of cattle in different rearing places, public health awareness including safe and hygienic practices while handling the cattle would be paramount importance in reducing the VTEC infections in humans.

ACKNOWLEDGEMENTS

The authors are highly thankful to Indian Council of Agricultural Research, New Delhi and Dean, College of Veterinary Science and Animal Husbandry, Uttar

Pradesh Pandit Deen Dayal Upadhayay Pashu Chikitsa Vigyan Vishvidhyalaya Ewam Go-Anusandhan Sansthan (DUVASU), Mathura, U.P., India for providing necessary funds and facilities to carry out the investigations.

REFERENCES

Anita, Kumar, A., Verma, A.K., Gupta, M.K. and Rahal, A.. 2014. Multi drug resistant pathogenic *Escherichia coli* in water sources and Yamuna river in and around Mathura, India. *Pak. J. Biol. Sci.,* **17(4)**: 540-544.

Beutin, L., Geier, D., Zimmerman, S. and Karch, H. 1995. Virulence markers of Shiga-like toxin-producing *E. coli* strains from healthy domestic animals of different species. *J. Clin. Microbiol.,* **33**: 631-635.

Blanco, M., Blanco, J., Blanco, J.E. and Ramos, J. 1993. Enterotoxigenic, verotoxigenic and necrotoxigenic *Escherichia coli* isolated from cattle in Spain. *Am. J. Vet. Res*., **54**: 1446-1451.

Brien, O.A.D. and Holmes, R.K. 1987. Shiga and Shiga-like toxins. *Microbiol. Rev*., **51**: 206-220**.**

Croxen, M.A. and Finlay, B.B. 2010. Molecular mechanisms of *Escherichia coli* pathogenicity *Nat. Rev. Microbiol*., **8**: 26–38.

Dhama, K., Rajagunalan, S., Chakraborty, S., Verma, A.K., Kumar, A. and Tiwari, R., Kapoor, S. 2013. Food-borne pathogens of Animal origin diagnosis, prevention and control and their zoonotic significance- A review. *Pak. J. Biol. Sci.,* **16**(20): 1076-1085.

Diaz, S., Vidal, D., Herrera-Leon, S. and Sanchez, S. 2011. Sorbitol fermenting, b-glucuronidase-positive, Shiga toxin negative *Escherichia coli* O157:H7 in free-ranging red deer in South-Central Spain. *Foodborne Pathog. Dis*., **8**: 1313–1315.

Eriksson, E., Aspon, A., Gunnarsson and Vagsholm, I. 2005. Prevalence of verotoxin producing *Escherichia coli* (VTEC) O157 in Swedish dairy herds. *Epidemiol. Infect*., **133**: 349-358.

Ewing, W.H. 1986. The genus *Escherichia*, In: P.R. Edwards and W. H Ewing (ed), Edwards and Ewing's identification of enterobacteriaceae, 4 ed. Elsevier Science Publishing Co Inc., New York, pp. 93-122.

Gyles, C.M. and Fairbrother, J.M. 2010. *Escherichia coli* In: Gyles, C.L., Prescott, J.F., Thoen, C.O. (Eds.), Pathogenesis of bacterial infections in animals. Blackwell Publishing, Ames, pp. 267–308.

Hiruta, N., Murase, T. and Okamura, N. 2001. An outbreak of diarrhea due to multiple antimicrobial resistant shiga toxin producine *E.coli* O26:h7 In a nursery. *Epidemiol. Infect*., **127**: 221-227.

Johnson, R.P., Clarke, R.C. and Gyles, C.L. 1996. Growing concerns and recent outbreaks involving non-O157:H7 serotypes of verotoxigenic *Escherichia coli. J. Food Prot.,* **59**: 1112-1122.

Khurana, P. and Kumar, A. 2005. Occurance of verotoxic *E. coli* in faeces and milk of cattle. *Harayana Vet*., **44**: 83-85.

Kobayashi, H., Shimada, J., Nakazawa, M., Morozumi, T., Pohjanvirta, T., Pelkonen, S. and Yamamoto, K. 2001. Prevalence and characteristics of Shiga toxin-producing *Escherichia coli* from healthy cattle in Japan. *Appl. Environ. Microbiol*., **67**: 484-489.

Kumar, A., Verma, A.K., Sharma, A.K. and Rahal, A. 2013. Presence of extended spectrum Beta-lactamases producing alpha hemolytic *Escherichia coli* in yellow-wattled Lapwing (*Vanellus malabaricus*). *Asian J. Anim. Sci.,* **7**(2): 64-69.

Mailk, S., Kumar, A., Verma, A.K., Gupta, M.K., Sharma, S.D., Sharma, A.K., Rahal, A., 2013. Incidence and drug resistance pattern of collibacillosis in cattle and buffalo calves in western Uttar Pradesh in India. *J. Anim. Health Prod.,* **1(1)**: 15-19.

OIE, 2004. Verocytotoxigenic *Escherichia coli. In:* Word Organization for Animal Health, Pairs manual of diagnostic tests and vaccines for terrestrial animals, 5th edition.

Oporto, B., Esteban, J.I., Aduriz, G., Juste, R.A. and Hurtado, A. 2008. *Escherichia coli* O157:H7 and non- O157 Shiga toxin-producing *E. coli* in healthy cattle, sheep and Swine herds in Northern Spain. *Zoonoses Pub. Hlth.*, **55(2)**: 73-81.

Parul, Bist, B., Sharma, B. and Manjula. 2014. Prevalence of verotoxin producing *Escherichia coli* non O157 in diarrhoeic and healthy calves. One Health : Harnesting biotechnology for addressing veterinary and biomedical concerns on food safety, zoonoses and environment sustainability. 12th annual conference of IAVPHS, Guwahati, India. 245-46.

Paton, A.W. and Paton. 1998. Detection and Characterization of Shiga Toxigenic *Escherichia coli* by using multiplex PCR Assays for *stx*1, *stx*2, *eaeA*, enterohemorrhagic *E. coli hlyA*, *rfb* O111, and *rfb* O157. *J. Clin. Microbiol*., **36(2**): 598–602

Rabin, B. 1994. Prevalence of verotoxic *Escherichia coli* in man animals, food and pulic health significance. M.V.Sc thesis, Deemed University, IVRI, Izatnagar.

Rogerie, F., Marecat, A., Gambade, S., Dupond, F., Beaubois P. and Lange M. 2001. Characterization of Shiga toxin producing *E. coli* and O157 serotype isolated in France from healthy domestic cattle. *Int. J. Food Microbiol.,* **63**: 217–223.

Sanchez, S., Martínez, R., Rey, J., García, A., Blanco, J., Blanco, M., Blanco, J.E., Mora, A., Herrera-León, S., Echeita, A. and

Alonso, J.M., 2010. Pheno-genotypic characterisation of *Escherichia coli* O157:H7 isolates from domestic and wild ruminants. *Vet. Microbiol*., **142**(3-4):445-9**.**

Sanchez, S., Sanchez, D.S., Martinez, R., Llorente, M.T., Herrera-Leon, S. and Vidal, D. 2013. The new allelic variantof the subtilase cytotoxin (sub AB_2) is common among Shiga toxin producing *Escherichia coli* strains from large animals and their meat and meat products. *Vet. Microbiol*. **166**: 645-649.

Schouten, J.M., Van De Giessen, A.W., Frankena, K., De Jong, M.C.M. and Graat, E.A.M. 2005. *Escherichia coli* O157 prevalence in dutch poultry, pig finishing and veal herds and risk factors in dutch veal herds. *Prev. Vet. Med*., **70**: 1-15.

Wieczorek, K., Beutin L. and Osek J. 2011. Rare VTEC serotypes of potential zoonotic risk isolated from bovine hides and carcases. *Vet. Rec*., **168**: 80-82.